

**REMARKS**

Claims 1, 3-7, 10, 12-17, 19-21, 23-26, and 38-42 are pending in the application. Claims 40-42 are new claims. Claim 3 is cancelled, its subject matter having been incorporated into Claim 1. Claims 1, 3-7, 10, 12-17, 19-21, 23-26, and 38-39 were rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. Claims 1, 3, 5-7, 10, 12-17, 19-21, 23-26, and 38-39 were rejected under 35 U.S.C. § 112, first paragraph as allegedly not enabled by the disclosure. Claims 1, 5, 6, 10, 12-17, and 23-25 were rejected under 35 U.S.C. § 102(e) as allegedly anticipated by U.S. Publication No. 2003/0166062 to Gonzalez-Villasenor (“Gonzalez-Villasenor”). Claims 1, 5, 6, and 12-14 were rejected under 35 U.S.C. §102(b) as allegedly anticipated by U.S. Patent No. 5,912,327 to Li et al. (“Li”). Claims 1, 5-7, 12-14, 16, 17, and 23-26 were rejected under 35 U.S.C. §102(b) as allegedly anticipated by the article entitled “Optimization of Inclusion Body Solubilization and Renaturation of Recombinant Human Growth Hormone from *Escherichia coli*” in Protein Expression and Purification (18, 182-192, 2000) by Patra et al. (“Patra”). Claims 1, 5, 6, 12-14, 16, 17, 23, and 25 were rejected under 35 U.S.C. §102(b) as allegedly anticipated by the article entitled “Kinetics of inclusion body production in batch and high cell density fed-batch culture of *Escherichia coli* expressing ovine growth hormone” in J. Biotechnology (75, 161-172, 1999) by Panda et al. (“Panda”). Claims 3, 4, 19-20, 26, 38, and 39 were rejected under 35 U.S.C. 103(a) as being allegedly obvious from Panda combined with U.S. Patent No. 4,810,643 to Souza (“Souza”), and further in view of U.S. Patent No. 5,618,927 to Ambrosius et al. (“Ambrosius”), U.S. Patent No. 5,773,581 to Camble et al. (“Camble”), and U.S. Publication No. 2002/0009798 to Pelleymounter et al. (“Pelleymounter”). Claim 21 was rejected under 35 U.S.C. §103(a) as allegedly being obvious from Patra combined with U.S. Patent No. 6,677,139 to Donnelly et al. (“Donnelly”).

Claims 1, 21, 26, and 38 are amended to more particularly point out and distinctly define the claimed subject matter. Support for the amendments may be found throughout the application and in the original claims, for example in Claim 3, and on pages 5-6, page 7, and page 15 of the specification. Claims 40-42 are new dependent claims. Support for the claims may further be found on pages 10 (Claim 40) and 11 (Claim 41-42) of the specification. No new matter is added into the case by any of these amendments or new claims.

Each and every rejection of the claims and/or specification is respectfully traversed, and favorable reconsideration is requested in view of the foregoing amendments and the following remarks.

A. Claims 1, 3-7, 10, 12-17, 19-21, 23-26, and 38-39 are Not Indefinite.

Claims 1, 3-7, 10, 12-17, 19-21, 23-26, and 38-39 are said to be indefinite under § 112, second paragraph. Independent Claim 1 is directed to a process for the making a biologically active protein, and Claims 3-10, 12-17, 19-21, 23-26, and 38-39 depend, directly or indirectly, from Claim 1.

By way of a summary, in accordance with one embodiment of the claimed invention, unicellular organisms are cultured such that protein therein is expressed and accumulated in non-classical inclusion bodies in relatively high amounts in the cell. The non-classical inclusion bodies are isolated from the cells. The protein is then solubilized from the non-classical inclusion bodies under non-denaturing conditions and then purified in a biologically active form, without requiring denaturation and renaturation steps previously considered essential.

It is alleged that the metes and bounds of Claim 1 could not be adequately determined due to the recitation of “cultivated organism having one or more cells”, which is said to be unclear. The Office Action further alleges that the recitation of “inclusion bodies having an aqueous solubility in the cells of the organism” in Claim 1 is a contradiction in terms and, thus, the metes and bounds of the claim are not able to be determined with sufficient precision.

Also, it is alleged that Claim 1 is indefinite because, in the Examiner’s view, it is unclear how fermentation can be regulated so as to perform the method step claimed. In addition, the Office Action contends that the recitation of “an agent capable of causing stress” in Claim 1 renders the claim indefinite because it is “not known if it [the agent] actually is causing stress.”

The Examiner further alleges that the term “substantially” in Claims 1 and 26 is a relative term which renders the claims indefinite. However, Applicants assert that the term “substantially” as used in Claims 1 and 21 is not indefinite when considered in context. A person of ordinary skill in the art would be sufficiently apprised as to the meaning and scope of the claimed subject matter by use of the term “substantially” throughout the specification, and Applicants’ clear indication the protein need not be 100% correctly folded in the practice of the claimed method. Furthermore, use of the term “substantial” in claims is well established by the

courts to be sufficiently definite when it is reasonable clear from the specification (as it is here) that the referenced parameter or feature is not required to be met exactly. See e.g., *In re Mattison*, 509 F.2d 563, 184 USPQ 484 (CCPA 1975) and *Andrew Corp. v. Gabriel Electronics*, 847 F.2d 819, 6 USPQ2d 2010 (Fed. Cir. 1988).

While not acknowledging that any of the present claims are indefinite, in the interest of advancing prosecution of the case, Applicants offer several amendments to address alleged deficiencies of in the claims. It is believed the claims are now sufficiently definite under 35 U.S.C. §112, and that a person of ordinary skill in the art reading the claims would be reasonably well apprised of the metes and bounds of the claimed subject matter.

Turning now to the alleged objectionable phrases cited in the rejection, Applicants respectfully remind the Examiner that the claims must be read in light of the specification (MPEP 2111 and 37 C.F.R. 1.75(d)(1)), and that Applicants may be their own “lexicographer” (MPEP 2111.01(IV)). The phrase “cultivated organism having at least one cell” simply means the cultivated organism is unicellular. It is plain from reading the specification that host organisms suitable for use in the presently claimed process are unicellular, such as bacteria or yeasts (page 4, last paragraph, and page 12, second full paragraph).

The amended claim now clarifies that the inclusion bodies are “non-classical”, as defined in the specification on page 7, fourth full paragraph. Regarding the “principle of performing the fermentation”, pages 13-14 of the specification teach the meaning of this phrase and how the same may be regulated. With regard to the phrase “an agent capable of causing stress”, it is believed that the amended claim no longer has the alleged deficiency. Accordingly, amended Claim 1 is not indefinite, and reconsideration and allowance of the claim are respectfully requested.

Claims 3-7, 10, 12-17, 19-21, 23-26, and 38-39 are said to be indefinite for depending from an allegedly indefinite base claim. However, it has been shown above that the base claim, at least as amended, is sufficiently definite. Hence, the claims dependent thereupon should also be deemed compliant with §112 since their alleged §112 problems were said to stem only from the fact of their dependancy. Accordingly, reconsideration and allowance of Claims 3-7, 10, 12-17, 19-21, 23-26, and 38-39 are also respectfully requested.

B. Claims 1, 3, 5-7, 10, 12-17, 19-21, 23-26, and 38-39 Are Enabled By the Disclosure.

Claims 1, 3, 5-7, 10, 12-17, 19-21, 23-26, and 38-39 are said to lack sufficient enablement for proteins other than G-CSF. Applicants respectfully disagree. One need not provide an example for each and every protein that may fall under the scope of a claim when there is an apparent commonality between the structures of the proteins such that a person of ordinary skill in the art would have a reasonable expectation of success when applying the disclosed process to such proteins.

In the present case, on page 4, last paragraph, structural characteristics of proteins suitable for use in the claimed process are described (i.e., hydrophobicity and few disulfide bridges). Further guidance is given on page 12, first full paragraph, and a reasonable expectation of success in using the claimed process for proteins other than G-CSF is established on page 9, last paragraph.

It is also evident from the specification that not every biologically active protein may be suitable for use with the claimed process, and one of ordinary skill in the art would be reasonably well apprised of appropriate selection criteria for practicing the claimed method for particular proteins other than G-CSF. In fact, a list of other suitable proteins is provided on pages 4 and 12, and claimed in Claim 1. In this case, since the recited proteins share the aforementioned common structural similarities, factors involved in the expression and isolation of such proteins are known from the clear teaching of the specification. Hence, Applicants assert that the single example provided provides sufficient enablement (MPEP 2164.03).

Accordingly, the claims are more than sufficiently enabled by the disclosure. Applicants believe that all requirements of 35 U.S.C. §112 are met by the claims. Reconsideration and allowance of claims 1, 5-7, 12-17, 19-21, 23-26, and 38-39 are hereby respectfully requested.

C. Claims 1, 5, 6, 10, 12-17, and 23-25 Are Not Anticipated By the Cited Reference.

Claims 1, 3-6, 12-15, 17, 23, 24, and 26 are said to be anticipated by Gonzalez-Villasenor. Claim 1 is an independent claim, from which the remaining claims depend. A reference can only properly be said to anticipate a claim if it contains within its four corners each and every limitation called for in the claim. From MPEP 2131, "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814

F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). See also *In re Donohue*, 226 USPQ 619 (Fed. Cir. 1985).

Independent Claim 1 calls for a process for the production of a biologically active protein that includes, among other things, the expression of the protein as a heterologous protein in an expression system. The expression system is a cultivated organism having at least one cell, and the protein is expressed as a substantially correctly folded protein precursor, having an aqueous solubility, in non-classical inclusion bodies. Also called for is the regulation of one or more cultivation parameters selected from the group consisting of temperature of cultivation, composition of cultivation medium, induction mode, principle of performing the fermentation, addition of a stress induction agent, and co-expression of auxiliary proteins.

The regulation of the one or more cultivation parameters increases the proportion of substantially correctly folded protein precursor present in the non-classical inclusion bodies in the cells, relative to the proportion of substantially correctly folded protein precursor present in inclusion bodies in cells of an organism not cultivated by regulating said parameters. The claim further calls for the isolation of the non-classical inclusion bodies from cells of the organism and optionally washing the non-classical inclusion bodies.

The process also includes solubilizing the substantially correctly folded protein precursor from the inclusion bodies under non-denaturing conditions by contacting the inclusion bodies with a non-denaturing solvent having a pH of about 8.0, and purifying the biologically active protein from the solubilized substantially correctly folded protein precursor and non-denaturing solvent. The claimed process is free from any steps of denaturation and renaturation of the protein. The protein is selected from the group consisting of G-CSF, GM-CSF, M-CSF, EGF, HAS, DNase, FGF, TNF-alpha, TNF-beta, interferons, and interleukins.

Gonzalez-Villasenor does not teach production of any of the recited proteins. Instead the reference teaches the production of troponin, fish somatotropin, and fish prolactin by a process completely different from the process called for in Claim 1. For example, Gonzalez-Villasenor fails to meet the limitation of contacting inclusion bodies with a non-denaturing solvent having a pH of about 8.0. Instead, Gonzalez-Villasenor uses denaturing solubilization solutions having considerably higher or lower pH values.

For at least these reasons, Gonzalez-Villasenor fails to anticipate independent Claim 1, and thus also dependent Claims 3-6, 12-15, 17, 23, 24, and 26. Reconsideration and allowance of Claims 1, 3-6, 12-15, 17, 23, 24, and 26 are hereby respectfully requested.

D. Claims 1, 5, 6, and 12-14 Are Not Anticipated By the Cited Reference.

Claims 1, 5, 6, and 12-14 are said to be anticipated by Li. The summary of the presently claimed process from Part C above is incorporated by reference herein.

Li is directed toward methods for solubilizing and purifying proteins expressed in an insoluble form using low concentrations of chaotropic agents, and methods for refolding the solubilized proteins. Li teaches solubilization of the protein using solutions having either higher or lower pH than required by the present claims.

Hence, Li fails to disclose the claimed limitation of “solubilizing the substantially correctly folded protein precursor from the inclusion bodies under non-denaturing conditions by contacting the inclusion bodies with a non-denaturing solvent having a pH of about 8.0.” Therefore, for at least this reason Li does not anticipate amended Claim 1, or dependent Claims 5, 6, and 12-14. Reconsideration and allowance Claims 1, 5, 6, and 12-14 are hereby respectfully requested.

E. Claims 1, 5-7, 12-14, 16, 17, and 23-26 Are Not Anticipated By the Cited Reference.

Claims 1, 5-7, 12-14, 16, 17, and 23-26 are said to be anticipated by Patra. The summary of the presently claimed process from Part C above is incorporated by reference herein.

Patra is directed toward solubilization of r-hGH (recombinant Human Growth Hormone) from inclusion bodies using 100 mM Tris buffer and 2M urea at pH 12.5. Accordingly, the disclosure of Patra does not describe every limitation of the presently amended claims. Specifically, Patra fails to disclose the claimed limitation of “solubilizing the substantially correctly folded protein precursor from the inclusion bodies under non-denaturing conditions by contacting the inclusion bodies with a non-denaturing solvent having a pH of about 8.0.” Among other things, Patra teaches the use of higher pH conditions to achieve the desired solubilization.

Therefore, for at least the above reason, Patra does not anticipate amended Claim 1, or dependent Claims 5-7, 12-14, 16, 17, and 23-26. Reconsideration and allowance of the amended claims are hereby respectfully requested.

F. Claims 1, 5, 6, 12-14, 16, 17, 23, and 25 Are Not Anticipated By the Cited Reference.

Claims 1, 5, 6, 12-14, 16, 17, 23, and 25 are said to be anticipated by Panda. The summary of the presently claimed process from Part C above is incorporated by reference herein.

Panda is directed toward a process for maximizing the volumetric productivity of recombinant ovine growth hormone (r-OGH) expressed in *E. coli*. Panda teaches solubilization of the protein from inclusion bodies using 50 mM Tris buffer (pH 10) containing 1% SDS. Accordingly, Panda fails to disclose, among other things, the claimed limitation of “solubilizing the substantially correctly folded protein precursor from the inclusion bodies under non-denaturing conditions by contacting the inclusion bodies with a non-denaturing solvent having a pH of about 8.0.”

Hence, for at least the above, reason Panda does not anticipate amended Claim 1, or dependent Claims 5, 6, 12-14, 16, 17, 23, and 25. Reconsideration and allowance of the claims are hereby respectfully requested.

G. Claims 3, 4, 19-20, 26, 38, and 39 Are Patentable Over the Cited References.

Claims 3, 4, 19-20, 26, 38, and 39 are said to be unpatentable over Panda in view of Souza, and further in view of Ambrosius, Camble, and Pelleymounter. The claims all depend from Claim 1, which was shown in part F above to be patentable over Panda under section 102.

In an attempt to remedy the deficiencies of Panda, the Examiner combines Panda with Souza, Ambrosius, Camble, and Pelleymounter. However, for at least the reason that none of the cited references discloses the claimed limitation (from independent Claim 1) of “solubilizing the substantially correctly folded protein precursor from the inclusion bodies under non-denaturing conditions by contacting the inclusion bodies with a non-denaturing solvent having a pH of about 8.0”, the combination cannot be said to render Claim 1 obvious.

Accordingly, reconsideration and allowance of Claims 3, 4, 19-20, 26, 38, and 39 are hereby respectfully requested.

H. Claim 21 is Patentable Over the Cited References.

Claim 21 is said to be unpatentable over Patra in view of Donnelly. However, Claim 21 is dependent upon independent Claim 1, which was shown in part E above to be patentable over Patra. In order for a claim to be obvious over a combination of references, it must be shown,

among other things, that the references contain at least all of the limitations of the rejected claim.

It must also be shown that it would have been obvious to a person of ordinary skill in the art at the time the invention was made to put the references together in the manner set forth in the Office Action to arrive at the allegedly obvious claim.

Neither requirement is met here. Specifically, neither Patra nor Donnelly teach or suggest the limitation (from Claim 1) of “solubilizing the substantially correctly folded protein precursor from the inclusion bodies under non-denaturing conditions by contacting the inclusion bodies with a non-denaturing solvent having a pH of about 8.0”, and nothing in the combination teaches this step carried out under these conditions. Even if the combination somehow contained this step, which it does not, nothing teaches combining these references in a way so as to provide a method containing this step.

Accordingly, since the combined references fail to render the independent claim unpatentable, dependent Claim 21 patentably distinguishes over the cited combination. Hence, reconsideration and allowance of Claim 21 are hereby respectfully requested.



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## CONCLUSION

Applicants respectfully submit that a full and complete response to the Office Action is provided herein, and that the application is now fully in condition for allowance. Action in accordance therewith is respectfully requested.

In the event this response is not timely filed, Applicants hereby petition for the appropriate extension of time and request that the fee for the extension along with any other fees which may be due with respect to this paper be charged to our Deposit Account No. 12-2355.

Respectfully submitted,

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